IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	§	
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Joseph KANNER et al	§	
	§	
Serial No.: 10/661,606	§	
	§	
Filed: September 15, 2003	§	Group Art Unit: 1655
	§	
For: INCREASING BIOAVAILABILITY OF CAROTENOIDS	§	
	§	
	§	Attorney Docket: **25629**
Examiner: Ralph J Gitomer	§	

Commissioner for Patents
P. O. Box 1450
Alexandria VA 22313-1450

DECLARATION OF PROF. JOSEPH KANNER UNDER 37 CFR 1.132

I am presently employed as Head of the Food Chemistry and Biochemistry Lab at The Volcani Center Bet-Dagan, where I am a Full Professor. I received my Ph.D. degree from the Hebrew University in 1974, worked as a post-doctoral fellow in MIT Boston, and was a visiting Professor at Cornell University. A current curriculum vitae is enclosed.

My research focuses on oxidation and anti-oxidation in food and nutrition. Since the beginning of my career, I have published more than 80 scientific articles in highly regarded journals and books, and have presented my achievements at many international scientific conferences.

I am a co-inventor of the subject matter claimed in the above-referenced U.S. patent application.

I have read the Official actions issued with respect to the above-identified application.

In this Official action, the Examiner has rejected claims 95, 96, 98, 100-101, 103, 109-111, 120 under 35 U.S.C. § 102(a) and 103(a) as being unpatentable over Breithaupt (Z Naturforsch). The Examiner has asserted that the extraction of deesterified carotentoids with ethyl acetate, in alkaline pH conditions, is within the

purview of the practicing artist, and thus no criticality is seen in the selected pH ranges. It is my belief that the methods of extraction used by Breithaupt are significantly less efficient than, and a such unsuitable for, the methods of the instant invention.

The Table enclosed herewith describes experimental results obtained by the present inventors illustrating the superior and unexpectedly efficient extraction of total, and highly deesterified carotenoids from whole homogenized red pepper tissue extracted by the mild alkaline extraction, compared to that of the same material extracted by the methanol/ethyl acetate/light petroleum (1:1:1, pH 7.4) solvent of Breithaupt.

These results conclusively prove that methods for extraction and deesterification of carotenoids, comprising alkaline ethyl acetate extraction of the carotenoids, as described and claimed in the instant application do indeed provide unexpected and significantly superior results as compared to the prior art methods, thereby overcoming the rejections of claims 95, 96, 98, 100-101, 103, 109-111, 120 under 35 U.S.C. § 102(a) and 103(a).

Ethyl acetate vs methanol/ethyl acetate/light petroleum (1:1:1) extraction of deesterified carotenoids:

Following deesterification with lipase, the carotenoid fraction was extracted by ethyl acetate, under alkaline pH, and methanol/ethyl acetate/light petroleum (1:1:1, pH 7.4). For alkaline ethyl acetate extraction, the lipase reaction mixture was made alkaline with NaOH, to pH 9.5 (with 0.1N NaOH solution), and ethyl acetate was then added at a ratio of 1 volume ethyl acetate to 5 volumes aqueous deesterification mixture, the phases mixed and allowed to separate. For methanol/ethyl acetate/light petroleum (1:1:1, pH 7.4) extraction, the solvents were added to the lipase reaction mixtures at a ratio of 1 volume solvents to 5 volumes aqueous deesterification mixture, the phases mixed and allowed to separate.

The aqueous phase was removed, the solvent phase reextracted four times with distilled water (water:solvent = 4:1 volume /volume), followed by drying by addition of superfluous sodium sulfate. The remaining solvent was then evaporated under vacuum in a Rotovapor (Buchi), and the resulting extracted oleoresin analyzed by HPLC, as described in the instant specification.

Treatments	MEOH: petrol-ether: ETA (1:1:1)- (According to Breithaupt)	ETA (According to Kanner et al.)
Extraction efficiency (1:4, aqueous: solvent)	33%	90%
Extraction efficiency (1:2, aqueous: solvent)	21%	78%

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United states Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

March 28, 2006

Joseph Kanner

Prof. Joseph Kanner
ARO, Volcani Center,
Department of Food Science
Enc.:
CV of Joseph Kanner

Joseph Kanner **September 2005**

CURRICULUM VITAE

I. Personal

Born at 1940
Married + 1

II. University Education and Additional Training

1961-1963	B.Sc., Faculty of Agriculture, Hebrew University of Jerusalem.
1965-1967	M.Sc., Faculty of Agriculture, Hebrew University of Jerusalem.
1970-1974	Ph.D. Thesis submitted to the Hebrew University of Jerusalem.
1974-1975	Post-doctorate at the Department of Nutrition and Food Science, M.I.T., Cambridge, U.S.A.
1981-1982	Sabbatical as Visiting Professor at the Department of Food Science, Cornell University, Ithaca, U.S.A.
1991-1992	Sabbatical as Visiting Professor at the Department of Food Science and Technology, University of Cal. Davis, U.S.A.

III. Positions Held and Academic Status

1968-1976	Research Scientist in Food Chemistry and Technology.
1976-1979	Head of research unit in Chemistry and Biochemistry of Citrus Products.
1976- to date	Head, unit of Food Chemistry and Biochemistry.
1985-1992	Head, Food Science Department, ARO, Volcani Center.
1989-to date	Professor adjunct, Department of Food Science. The Hebrew University, Faculty of Agriculture, Rehovot.
1995-1998	Director of the Institute for Technology & Storage of Agricultural Products.

IV. Teaching and Training Experience (including tutorship of students)

1976-1978	Lecture of the course on Chemistry of Foods, at the Agricultural High School, Rupin
1979	Teaching the course on Chemistry and Biochemistry of Foods, at the Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot.
1979	Teaching the course on Analysis and Food Chemistry, at the Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot.

To date, 21 graduate students of the Hebrew University have carried out their M. Sc. thesis under my sopervision:

Professional and Academic Status

1990-to date Researcher Scientific Grade A+ (equivalent to full Professor).

Functions in Professional Societies

1978-1980 Chairman of the Israel Society for Food and Nutrition Sciences, affiliated to the I.F.T.

1993-to date Committee Member of the Israel Society for Oxygen and Free Radicals Research.

1999- President elected of the Israel Society for Oxygen and Free Radicals Research.

VIII. Membership in Professional Societies

Member of the Israel Society for Food and Nutrition Science.
Member of the I.F.T. Society.
Member of the Society for Free Radical Research.

IX. Contribution to the Scientific Community

During my career as a researcher, Department Head and Director of the Institute and as the Chairman of two societies, I organized many seminars and symposia in the area of food science and free radical research.

XI. Awards and honors.

2001 ISI honors for "Highly Cited Researcher" (1981-2001) in recognition for outstanding achievements and contributions to the international research community and one of the most influential researcher in the field of Agriculture.

2002 The Israel Academy of Science found our papers in the field of Chemistry to be the most highly cited in Israel (between the first 200 papers) for the years (1981-2001).

XII. LIST OF PUBLICATION

A. Reviewed articles.

1.a Hershkovitz, E. and Kanner J. The effect of heat treatment of β-glucosidase activity in canned whole apricots. J. Food Technol. 1970, 5: 197-201.

2.b Palevith, D., Harel, S., Kanner, J. and Ben-Gera, I. The effect of preharvest sweet dehydration on the composition of once-over harvest sweet paprika. Sciential Horticulture. 1975, 3: 143-148.

3.a Kanner, J., Mendel, H. and Budowski, P. Carotene oxidizing factors in red pepper fruits (Capsicum annum L.): Ascorbic acid. J. Food Sci. 1976, 41: 183-185.

4.a Kanner, J. and Harel, M. Changes in lysozyme due to reactions with peroxidizing methyl linoleate in dehydrated model system. J. Agric. Food Chem. 1976, 24: 486-472.

5.a Kanner, J., Mendel, H. and Busowski, P. Prooxidant and antioxidant effects of ascorbic acid and metal salts in a β-caroatene-linoleate model system. J. Food Science. 1977, 42: 60-64.

6.a Kanner, J., Mendel, H. and Budowski, P. Carotene oxidizing factors in red pepper fruits (Capsicum annum L.): Peroxidase-like protein. J. Food. Sci. 1977, 42: 1549-1551.

7.a Kanner, J., Harel, S., Palevitch, D. and Ben-Genra, I. Color retention in sweet red paprika powder as affected by moisture contents and ripening stage. J. Food Technol. 1977, 12: 59-64.

8.b Juven, B.J., Kanner, J. and Weisslovitz, H. Influence of orange juice composition on the thermal resistance of spoilage yeasts. J. Food Sci. 1978, 43: 1074-1076.

9.a Kanner, J. and Bodowski, P. Carotene oxidizing factors in red pepper fruits (Capsicum annum L.): β-carotene-linoleic acid solid model. J. Food Sci. 1978, 43: 524-526.

10.a Kanner, J., Mendel H. and Budowksi, P. Carotene oxidizing factors on red pepper fruits (Capsicum annum L.): Oleoresin-cellulose solid model. J. Food Sci. 1978, 43: 709-712.

11.a Harel, S., Kanner, J., Juven, B.J. and Golan, R. Long-term preservation of high-moisture dried apricots with and without chemical preservatives. Lebensm-Wiss. M-Technol. 1978, 11: 219-221.

12.a Kanner, J., Elmaleh, H., Reuveni, O. and Ben-Gera, I. Invertase (β-fructofuranosidase) activity in three date cultivars. J. Agric. Food Soc. 1978, 27: 1238-1240.

13.a Kanner, J. S-nitrosocysteine (RSNO), and effective antioxidant in cured meat. J. Am. Oil Chem. Soc. 1979, 56: 74-76.

14.a Kanner, J., Harel, S. and Mendel, H. Content and stability of α-tocopherol in fresh and dehydrated pepper fruits (Capsicum annum L.). J. Agric. Food Chem. 1979, 27: 1316-1318.

15.a Kanner, J. and Juven, B.J. S-nitroso cysteine as an antioxidant, color-devloping and anti-clostridial agent in communited turkey meat. J. Food Sci. 1980, 45: 1105-1108 & 1112.

16.a Kanner, J. Nitric-oxide myoglobin as an inhibitor of lipid oxidation. Lipids, 1980, 15: 944-948.

17.c Angel, S., Basker, D., Kanner, J. and Juven, B.J. Assessment of shelf life of fresh water prawns stored at 0°C. J. Food Technol. 1981, 16: 357-366.

18.a Kanner, J., Harel, S., Fishbein, Y. and Shalom, P. Furfural accumulation in stored orange juice concentrates. J. Agric. Food Chem. 1981, 29: 948-950.

19.a Kanner, J. and Harel, S. Ascorbic acid oxidase in orange peel. J. Food Sci. 1981, 46: 1407-1409.

20.a Kanner, J., Ben-Shalom, N. and Shomer, I. Pectin-hesperidin interaction in a citrus cloud model system. Lebensm.-Wiss. u.-Technol. 1982, 15: 348-350

21.a Kanner, J., Fishbein, J., Shalom, P., Harel, S. and Ben-Gera, I. Storage stability of orange juice concentrate packages aseptically. J. Food Sci. 1982, 47: 429-431.

22.b Ben-Shalom, N., Shomer, I., Pinto, R. and Kanner J. Optimum conditions for determining depolimerization of pectic substances with Sumner reagent. Food Chemistry, 1982, 9: 205-212.

23.a Kanner, J. and Kinsella, J.E. Lipid determination initiated by phagocytic cells in muscle foods: β-carotene destruction by a myeloperoxidase-hydrogen peroxide-halide system. J. Agric. Food. Chem. 1983, 31: 370-376.

24.a Kanner, J. and Kinsella, J.E. Lipid determination: β-carotene destruction and oxygen evolution in a system containing lactoperoxidase, hydrogen peroxide-halide system. Lipids, 1983, 18: 198-203.

25.a Kanner, J. and Kinsella, J.E. Initiation of lipid peroxidation by a peroxidase-hydrogen peroxide-halide system. Lipids, 1983, 18: 204-210.

26.b Ben-Shalom, N., Shomer, I. And Kanner, J. Model system of natural citrus cloud caused by specific interaction of hesperidin with pectin. Lebensm.-Wiss. u.-Technol. 1983, 17: 125-128.

27.a Kanner, J., Harel, S., Shagalovich, J. and Berman, S. The antioxidative effect of nitrite in cured meat products. Nitric-oxide iron complexes of low molecular weight. J. Agric. Food Chem. 1984, 32: 512-515.

28.a Kanner, J. and Harel, S. Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. Arch. Biochem. Biophys. 1985, 237: 314-321.

29.a Kanner, J. and Harel, S. Lipid peroxidation and oxidation of several compounds by activated metmyoglobin. Lipids, 1985, 20: 625-628.

30.b Ben-Shalom, N., Pinto, R., Kanner, J. and Berman, J. A model system of natural orange juice cloud. Effect of calcium on hesperidin-pectin particles. J. Food Sci. 1985, 50: 1130-1132.

31.a Harel, S. and Kanner, J. Hydrogen peroxide generation in ground muscle tissues. J. Agric. Food Chem. 1985, 33: 1186-1188.

32.a Harel, S. and Kanner, J. Muscle membranal lipid peroxidation by initiated by H_2O_2 activated metmyglobin. J. Agric. Food Chem. 1985, 33: 1188-1192.

33.b Shomer, I., Lindner, P., Vasiliver, R., Kanner, J. and Merin, U. Colloidal fractions of citrus fruit aqueous peel extract. Lebensm.-Wiss. u.-Technol. 1985, 18: 357-365.

34.a Kanner, J., Harel, S. and Hazan, B. Muscle membranal lipid peroxidation

by an "Iron Redox Cycle" system: Initiation by oxyradicals and site-specific mechanism. J. Agric. Food Chem. 1986, 34: 506-510.

35.b Juven, B.J. and Kanner, J. The effect of ascorbic acid, isoascorbic acid and dehydroascorbic acids on the growth and survival of Compylobacter jejuni. J. Appl. Bacteriol. 1986, 61: 339-345.

36.a Kanner, J. and Harel, S. Desferrioxamine as an electron donor. Inhibition of membranal lipid peroxidation initiated by H_2O_2-activated metmyoglobin and other peroxidizing systems. Free Radical Res. Comms. 1987, 3: 309-317.

37.a Kanner, J. German, J.B., and Kinsella, E.J. Initiation of lipid peroxidation in biological systems. Crit. Rev. Food Sci. and Nutr. 1987, 25: 317-364.

38.c Naim, M., Shtrim, B., Kanner, J. and Peleg, H. Ferulic acid as a precursor of off-flavor in orange juice during storage. J. Food Sci. 1988, 53: 500-504.

39.a.Kanner, J., Shagalovich, I., Harel, S. and Hazan, B. Muscle lipid peroxidation dependent on oxygen and free metal ions. J. Agric. Food Chem. 1988, 36: 409-412.

40.a Kanner, J., Hazan, B. and Doll, L. Catalytic "Free" iron in muscle foods. J. Agric. Food Chem. 1988, 36: 412-415.

41.a Kanner, J., Sofer, F., Harel, S. and Doll, L. Antioxidant activity of ceruloplasmin in muscle membranal and in-situ lipid peroxidation. J. Agric. Food Chem. 1988, 36: 415-417.

42.a Harel, S. and Kanner, J. The generation of ferryl and hydroxyl radicals during interaction of hemeproteins with hydrogen peroxide. Free Rad. Res. Comms. 1988, 5: 11-20.

43.a Harel, S., Salan, M.D. and Kanner, J. Iron release from metmyoglobin, methemoglobin and cytochrome c by a system generating H_2O_2. Free Rad. Res. Comms. 1988, 5: 21-34.

44.b Juven, B.J., Kanner, J., Weisilowicz, H. and Harel, S. Effect of ascorbic acid and isoascorbic acid on the survival of C. jejuni in poultry meat. J. Food Prot. 1988, 51: 436-437.

45.a Harel, S. and Kanner, J. Hemoglobin and myoglobin as inhibitors of hydroxyl radicals generation in a model system of "Iron Redox" cycle. Free Rad. Res. Comms. 1989, 6: 1-10.

46.a Kanner, J., Bartov, I., Salan, M.O. and Doll, Linda. Effect of dietary iron level on in-situ turkey muscle lipid peroxidation. J. Agric. Food Chem. 1990, 38: 601-604.

47.a Kanner, J., Salan, M.A., Harel, S. and Shegalovich, I. Lipid peroxidation of muscle food: The role of the cytosolic fraction. J. Agric. Food Chem. 1991, 39: 242-246.

48.a Kanner, J. and Doll, Linda. Ferritin in turkey muscle tissue: A source of catalytic iron ions for lipid peroxidation. J. Agric. Food Chem.

1991, 39: 247-249.

49a. Kanner, J., Harel, S. and Jaffe, R. Lipid peroxidation of muscle food as affected by NaCl. J. Agric. Food Chem. 1991, 39: 1017-1021.

50.a Kanner, J., Granit, R. and Akiri, B. Nitric oxide, an inhibitor of lipid oxidation by lypoxygenase cycloxygenase and hemoglobin. Free Radic. Biol. and Med. 1990, 9(S1): 143.

51.a Kanner, J., Harel, S. and Granit, R. Nitric oxide as an antioxidant. Arch. Biochem. Biophys. 1991, 289: 130-136.

52.a Kanner, J., Harel, S. and Granit, R. Nitric oxide, an inhibitor of lipid oxidation by lipoxygenase cycloxygenase and hemoglobin. Lipids, 1992, 27: 46-49.

53.a Kanner, J. and Rosenthal, I. An assessment of lipid oxidation in foods. Pure Appl. Chem. 1992, 64, 1959-1963.

54.a Frankel, EN. Kanner J. German, B. Parks, E. And Kinsella E.J. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet, 1993, 341: 454-457.

55.b Ittah, Y., Kanner, J. and Granit, R. Hydrolysis study of carotenoids pigments of paprika (Capsicum annum L. variety Lehava) by HPLC/Photodiode Array detection. J. Agric. Food Chem. 1993, 41: 899-901.

56.b Kinsella, E.J., Frankel, E., German, B. and Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol. 1993, 47:L 85-89.

57.b Miller, D.K., Gomez-Besauri, J.V., Smith V.L., Kanner, J. and Miller, D.D. Dietary iron in swine reactions affects nonheme iron and TBARS in pork skeletal muscles. J. Food Sci. 1994, 59: 747-750.

58.c Miller, D.K., Smith, V.L., Kanner, J. Miller D.D. and LowLess H.T. Lipid oxidation and warmover aroma in cooked ground pork from swine fed increasing levels of iron. J. Food Sci. 1994, 59: 751-756.

59.a Kanner, J. Frankel, E. Granit, R., German, B. and Kinsella, JE. Natural antioxidants in grapes and wines. J. Agric. Food Chem. 1994, 42: 64-69.

60.c Frankel, EN. Huang, SW. Kanner, J. and German, B. Interfacial Phenomena in the evaluation of antioxidants: Bulk Oils versus emulsions. J. Agric. Food Chem. 1994, 42: 1054-1059.

61.b Juven, B.J., Kanner, J., Schved, F. and Weisslowicz, H. Factor that interact with the antibacterial action of thyme essential oil and its active constituents. J. Appl. Bacteriol. 1994, 76: 626-631.

62.a Kanner, J. Oxidative processes in meat and meat products: Quality implication. Meat Science, 1994, 36: 169-189.

63.b Philosoph-Hadas, S., Meir, S., Akiri, B. and Kanner, J. Oxidative defence systems in leaves of three edible herb species in relation to their senescence rates. J. Agric. Food Chem. 1994, 42, 2376-2381.

64.a Levy, A. Harel, S. Palevich, D. Akiri, B. Managem, E. and Kanner, J. Crotenoid pigments and β-carotene in paprika fruit (Capsicum spp.) with different genotypes. J. Agric. Food Chem. 1995, 43: 362-367.

65.b Meir, S. Kanner, J. Akiri, B. and Philosoph-Hadas, S. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. J. Agric. Food Chem. 1995, 43: 1813-1819.

66.b Bartov, I. and Kanner, J. Effect of high level of dietary iron, iron injection, and dietary vitamin E on the oxidative stability of turkey meat during storage. Poultry Sci. 1996, 75: 109-112.

67.a Kanner, J. Nitric oxide and metal-catalyzed reactions. Methods in Enzymology. 1996, 269: 218-229.

68.a Lapidot, T., Harel, S., Granit, R., and Kanner, J. Bioavailability of red wine anthocyanins as detected in human urine. J. Agric. Food Chem. 1998, 46: 4297-4302.

69.a Lapidot, T., Harel, S. Akiri, B. Granit, R. and Kanner, J. pH-Dependent forms of red wine anthocyanins as antioxidants. J. Agric. Food Chem. 1999, 47: 67-70.

70.b Weinberg, Z.G., Akiri, B. Putievsky, E. and Kanner, J. Enhancement of polyphenol recovery from rosemary (Rosmarinus officinalis) and sage (Salvia officinalis) by enzyme- assisted ensiling (ENLAC). J. Agric. Food Chem. 1999, 47: 2959-2962.

71.a Kanner, J., Karel, S. and Granit, R. Betalains – A new class of dietary cationized antioxidants. J. Agric. Food Chem. 2001, 49 5178-5185.

72.a Gorelik, S. and Kanner, J. Oxymyoglobin oxidation and membranal lipid peroxidation initiated by "iron-redox cycle". J. Agric Food Chem. 2001, 48, 5939-5944.

73.a Gorelik, S. and Kanner, J. Oxymyoglobin oxidation and membranal lipid peroxidation initiated by "iron-redox cycle": Prevention of oxidation by enzymic and non-enzymic antioxidants. J. Agric Food Chem. 2001, 48, 5945-5950.

74.a Granit, R. Angel, S. Akiri, B., Holzer, Z., Aharoni, Y., Orlov, A. and Kanner, J. Effects of vitamin E supplementation of lipid peroxidation and color retention of salted calf muscle from diet rich in polyunsaturated fatty acids. J. Agric Food Chem. 2001, 48, 5951-5956.

75.a Kanner, J. and Lapidot, T. The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. Free Radic. Biol. and Med. 2001, 31: 1388-1395.

76.a Lapidot, T., Walker, M.D., Kanner, J. Can apple antioxidants inhibit tumor cell proliferation? Generation of H_2O_2 during interaction of phenolic compounds with cell culture media. J. Agric. Food Chem. 2002, 50, 3156-3160.

77.a Lapidot, T., Walker, M.D., Kanner, J. Antioxidant and prooxidant effects of

phenolics on pancreatic β-cells in vitro. J. Agric. Food Chem. 2002, 50, 7220-7225.

78.a Lapidot, T.,Granit, R., and Kanner, J.Lipid peroxidation by "free" iron and myoglobin as affected by dietary antioxidants in simulated gastric fluids. J. Agric. Food Chem. 2005, 53, 3383-3390.

79.a Lapidot, T.,Granit, R., and Kanner, J.Lipid hydroperoxidase activity of myoglobin and phenolic antioxidants in simulated gastric fluid. J. Agric. Food Chem. 2005, 53, 3391-3396.

80.a Gorelik, S., Lapidot, T., Shacham, I., Granit, R., Ligumsky, M., Kohen, R. and Kanner, J. Lipid peroxidation and coupled vitamin oxidation in simulated and human gastric fluid inhibited by dietary polyphenols: health implications. J. Agric. Food Chem. 2005, 53, 3397-3402.